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Title: Military Working Dog Assessment II: The Use of an Open Field Model to Assess Sound-Associated Anxiety

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Abstract

Military Working Dogs used to detect improvised explosive devices (IED) in combat situations must be emotionally resilient to gunfire and other loud environmental sounds. Dogs that show anxiety responses to environmental stimuli may be behaviorally unsuited to further training and eventual deployment. In a novel application, this study presented candidate IED-detection dogs, male and female Labrador retrievers, with either ambient noise or recorded thunderstorm or gunfire sounds in a controlled open field arena. Behavioral and physiological responses to these stimuli were evaluated. Physiological data including post-stimuli heart rate, body temperature, and salivary cortisol concentrations were measured. Anxiety scores were calculated from video records by an observer blind to sound treatment. The results revealed that behavioral data were more sensitive than physiological data and dogs stratified with respect to anxiety scores, which ranged from subtle to profound. The results suggest that the open field sound test may be a useful method to evaluate dogs for suitability for training as IED-detection dogs.

Keywords
Military working dogs, open field, anxiety scores, thunderstorm, gunfire

1. Introduction

In simplest terms, anxiety is the anticipation of danger, usually from unknown or imagined origin. Fear is the awareness of immediate danger. Anxiety and fear may be difficult to differentiate behaviorally; the terms are often used interchangeably to describe a constellation of behavioral and physiological responses. Although in some cases, anxiety and fear may be adaptive and enhance survivorship, in other cases, anxiety and fear may impair an animal's function and inhibit learning. In severe cases and in stressful environments, an exaggerated maladaptive response may occur, leading to behavioral debilitation. Such a response would be devastating to military working dogs (MWDs) in combat situations.

Anxious MWDs may be hypervigilant even in the absence of specific stimuli and may startle easily, assume low posture, or show more subtle, but observable, signs of yawning, tongue flicking, or lip licking. Fear-induced physiologic responses resulting from enhanced activation of the hypothalamic–pituitary–adrenal (HPA) axis. Release of cortisol, noradrenalin, and adrenaline may also occur. Acute physiological responses include increased heart rate, respiration, and salivation. Prolonged stress-induced activation of the HPA axis is a known risk factor for certain gastrointestinal, dermatologic, immunologic, and urinary tract disorders in dogs (Beerda et al., 1999; Gue et al., 1987, Hydbring-Sandberg et al., 2004) and may represent a welfare concern for working dogs.

In dogs, the term "noise phobia" refers to the expression of excessive fear in response to a loud sound, and may be specific to thunderstorms or other auditory stimuli (Sherman and Mills 2008; Crowell-Davis et al., 2003). Although differences exist between a playback of a sound recording and the actual events of thunderstorms, fireworks, and other sound stimuli, investigators have successfully used audio recordings in controlled environments to reveal noise phobias and to provide an objective measure of an individual dog's reactions to these stimuli (Araujo et al., 2009; Araujo et al., 2010; Shull-Selcer and Stagg, 1991). These playback experiments have also been used to document the success of pharmacologic or other methods to attenuate fear responses (Overall, 2002).

The Open Field Test (OFT) has a long history of use in experimental psychology (Walsh and Cummins, 1976). In the restricted and controlled environment of an OFT, the behavioral responses of individual animals may be observed and quantified. Animal movement may be measured by infrared photobeams, electronic sensors, computer-based tracking systems, and other modalities. The OFT was originally developed to assess locomotor activity and anxiety in rodents, revealing that, when anxious, the natural tendency of rodents is to display thigmotaxis, orienting close to the walls of the enclosure and avoiding the center of the open field arena (Simon et al., 1994). The basic principles and design of the rodent OFT have been adapted for use in other species including dogs (Araujo et al., 2009; Siwak et al, 2003; Head et al, 1997). In an OFT, dogs display changes in locomotor activity and human approach/avoidance as a function of age-related cognitive decline (Siwak et al, 2003; Head et al 1997). They display anxiety responses to thunderstorm recordings, which may be attenuated by anxiolytic agents (Araujo et al, 2010).

This study utilized the OFT paradigm to evaluate anxiety-related behavioral responses to loud sounds by dogs selected for training as Improvised Explosive Device (IED) Detection Dogs

(IDD). IDDs are specifically trained to detect IEDs in combat zones. As such, they may be exposed to loud sounds, including rapid gunfire and explosives. The OFT may be used to evaluate dogs for their suitability to work with noise-induced stress and may help avoid premature discharge of MWDs for undesirable behavioral reasons (Evans et al, 2007). Our objective was to expose candidate IDDs to the sounds of thunderstorms and gunfire in an OFT, and to evaluate each dog's anxiety using physiologic measures, activity data, and subjective anxiety scoring.

2. Materials and Methods

2.1 Subjects

The experimental subjects were 16 Labrador retriever dogs between 2-4 years of age. There were 8 intact males, 5 intact females, and 3 spayed females. The dogs had been selected from field trial stock as candidates for IDD training by a private MWD training firm (K2 Solutions, Southern Pines, NC). Additional details regarding their selection, housing, and welfare oversight have been described (Sherman et al., 2013). At the time of open field testing, all dogs had been in residence for approximately 3.5 months in a dedicated indoor canine facility under veterinary supervision at the North Carolina State University (NCSU) College of Veterinary Medicine (CVM) Laboratory Animal Resources Unit. They were individually housed in kennels separate from the test areas, and were maintained on a stable regime of feeding, exercise, and rest. All procedures were approved by the NCSU Institutional Animal Care and Use Committee.

2.2 Open-field test (OFT) arena

The OFT arena consisted of a room approximately 2.9 X 2.7 m, located in a dedicated free-standing building, maintained at an ambient temperature of approximately 20-25°C (Figure 1). The OFT arena had an epoxy painted cement floor, and was constructed of three cement block walls and a fourth modular wall with door and viewing panel. The OFT arena was equipped with a hide (W61 x H76 x L91 cm), constructed of high-density polyethylene boards (King StarBoard®, King Plastic Corporation, North Port, FL), into which the dogs could retreat. Two cameras were mounted such that dogs could be visualized at all times while in the OFT arena. One camera was mounted overhead in the center of the ceiling, while a second horizontal camera was mounted outside the arena 0.5 m above the OFT floor. The horizontal camera was fitted with an infrared filter and illuminator and was directed through a camera port in an opaque window to record each dog's behavior while in the hide and adjacent areas. To reduce olfactory cues between subjects, the OFT arena was sanitized with Virkon®-S (Dupont, Fayetteville, NC) diluted to 0.25% strength, applied to the floor and allowed to air dry following each dog's session.

2.3 Sound stimuli and OFT procedures

Digital audio recordings of the sounds of a thunderstorm (CanCog Technologies, Toronto, Ontario) or simulated gun battle (K2 Solutions, Southern Pines, NC) were played through two overhead speakers in the OFT arena at standardized sound pressure levels (SPL), measured in decibels (dB). Background sound level (without a dog in the arena) was approximately 46-50 dB

SPL. The mean thunderstorm sound level used was 88.8 dB SPL; the peak level was 104 to 105 dB; the A-weighted sound exposure level (SEL) was 110.9 dBA. The mean gun battle sound level used was 95.2 dB; the A-weighted SEL was 117.2 dBA.

Open-field testing was completed during a two week period (8 dogs/week). Each week, testing was performed on each dog between 1300 to 1600 hours on five consecutive days (Monday-Friday). None of the subjects had previously been exposed to the OFT arena prior to testing. Within each group of 8 subjects, males were evaluated before females. Otherwise, the order of the dogs was initially randomized for each group (Week 1 or Week 2), and then dogs were tested in the same order each day.

The protocol was identical for all dogs, with the order and intensity of sound presentation standardized. Each dog was placed in the open field arena for a 9- minute session on 5 consecutive test days. Each 9- minute session was divided into three 3- minute periods (A, B, and C) as illustrated in Table 1. On Day 2, the sound of a thunderstorm was played at the standardized sound pressure level during period B, while on Day 4, the sound of gunfire was played at the standardized sound pressure level during period B.

2.4 Physiologic data

Physiological data, including heart rate and body temperature, were collected by a trained investigator immediately before and after each session for each dog on Days 1-5. Heart rate was collected by auscultation with a stethoscope over the heart base or by femoral pulse detection. Body temperature was collected via digital rectal thermometer. Saliva was also collected immediately after each session for cortisol analysis as described previously (Sherman et al, 2013). These end-of-exposure data were compared to mean salivary cortisol levels obtained between the hours of 1300 and 1600 on two separate days approximately one week prior to commencement of the testing protocol (baseline measurements). In brief, saliva was collected with a 15-cm piece of test-specific cotton rope (Salimetrics, State College, PA) by inserting one end of the rope into the dog's mouth for approximately 2 minutes. Dogs had been conditioned to accept the rope in their mouths in anticipation of a food reward following saliva collection. This facilitated easy acquisition of an adequate volume for analysis. Samples were extracted and stored at -20 °C until analysis using a high sensitivity salivary cortisol enzyme immunoassay kit (Salimetrics, State College PA).

2.5 OFT behavioral data analysis

Video recordings from the overhead camera were analyzed for motor activity in terms of distance traveled per time period using a dedicated behavioral analysis program (EthoVision XT 7.1, Noldus Information Technology, Leesburg, VA). Since dogs could be visualized at all times by the two cameras, there were no "out of sight" time intervals. A sampling rate of 10 samples per second was used during video acquisition. After acquisition but prior to calculations, the maximum track smoothing function was applied. The center-point/not moving duration parameter was manually calculated by setting start and stop velocity thresholds. A start velocity of 0.10 m/sec and a stop velocity of 0.07 m/sec were used.

2.6 Anxiety scores

Video recordings from both camera views were used to evaluate anxiety-associated behaviors and to generate anxiety scores. Videos were reviewed without sound by a single trained observer who remained blind to session and sound treatment. Anxiety scores were assigned for each 3-minute period over 5 days, a total of 15 periods per dog. Prior to the analysis, the 15 video recordings for all days were randomized. The anxiety scores were based on duration and intensity of specific anxiety-associated behaviors observed over a given period of time (Table 2). Scores were based on a scale of 1 through 6 increasing stepwise by half points, where a score of 1 reflected no expression of anxiety behaviors and a score of 6 reflected severe anxiety behaviors exhibited most of the time.

Anxiety scores were assigned on this scale in four categories of anxiety behavior for each 3-minute period (Table 3): negative (passive), positive (active), global (subjective intermediary of negative and positive scores), and mean global (calculated average of the negative and positive scores). Negative anxiety behaviors included decreased activity, lowered body postures, and autonomic /conflict behaviors. Positive anxiety behaviors included startling, vigilance, and active responses. Therefore, each dog had 4 scores for each 3 minute period (3 assigned by the observer and one calculated global mean) for a total of 12 anxiety scores for each 9 minute test session. Due to the similarity between the global and mean global scores, only the mean global score was used in the analysis.

2.7 Statistical Analysis

Data were analyzed by ANOVA and ANCOVA, with day, period, treatment, and sex evaluated as factors, and baseline values as covariates where appropriate. Because the group size for spayed versus intact female dogs was small, all female dogs were analyzed collectively irrespective of their reproductive status. When a factor was identified as not statistically significant, the data were pooled appropriately. We utilized SAS v9.2 (SAS, Cary, NC) for statistical analysis. The results were considered statistically significant if $p \leq 0.05$. All results are shown for all dogs (n= 16) unless otherwise specified.

3. Results

Day 1 was designed to acquaint dogs to the open field test arena as part of the acclimation process. As expected, responses on Day 1 were higher than all other days. Sessions on Days 3 and 5 were considered control days; no stimuli were presented. Thus, in subsequent analyses, the values on these two days were pooled and subsequently termed Days 3 & 5. These two days were evaluated to ensure that pooling was appropriate (no significant differences found between days) for each physiological measure. Treatment periods were Day 2, period B (Thunderstorm), and Day 4, period B (Gunfire).

One dog ('Hunter') became destructive in the OFT arena on the first day of testing only (Day 1). His open field test session on that day was terminated after 6 minutes. Therefore, for this dog, data are available for periods A and B only and physiological data were collected after 6 minutes rather than 9 minutes.

3.1 Physiologic data

In all dogs, heart rates and body temperatures were within normal physiologic ranges.

3.1.1 Heart rate. There was no difference between males and females for the change in heart rate over the session (post-pre) using an ANCOVA model controlling for day ($p=0.9081$), so data from males and females were pooled for further analysis of day effects. The final model analyzed change in heart rate as a function of pre-session heart rate and categorical day ($p=0.0277$) using an ANCOVA model. Mean heart rate changes by day are shown in Figure 2. The average change in HR on Day 1 was significantly different from Days 3 & 5 (ANCOVA, $p=0.0031$), but not significantly different from Day 2 (ANCOVA, $p=0.1887$) or Day 4 (ANCOVA, $p=0.0581$). Day 2 was not significantly different from Day 4 ($p=0.5495$) or from Days 3 & 5 ($p=0.1876$). On control days (Days 3 & 5), the heart rates at the end of the session were slightly lower than the heart rates prior to the start of the session (t-test, $p<0.001$). On sound treatment days (Days 2 and 4), the heart rates at the end of the session were not significantly lower (t-test, $p=0.3034$, and $p=0.0542$ respectively).

3.1.2 Body temperature. There was no difference between males and females for the change in temperature over the session (post-pre) using an ANCOVA model controlling for day ($p=0.0902$), so data from males and females were pooled for subsequent analysis of session effects. The final model analyzed change in temperature as a function of pre-session body temperature and 4-level day ($p=0.0376$) using an ANCOVA model. On all days, the dogs' post-session body temperatures were not different from the pre-session body temperatures. Mean body temperature changes by day are shown in Figure 3. The average change in body temperature on Day 1 was significantly greater than Day 2 (ANCOVA, $p=0.0236$) and Days 3 & 5 (ANCOVA, $p=0.0052$), but not Day 4 (ANCOVA, $p=0.0876$). Day 2 was not significantly different from Day 4 ($p=0.5745$) or from Days 3 & 5 ($p=0.8060$), and Day 4 was not significantly different from Days 3 & 5 ($p=0.3716$).

3.1.3. Salivary cortisol concentration. Although the salivary cortisol concentrations did not follow the trend of decreased response over days in the open field, there was a statistically significant effect of sex was seen for this parameter (ANCOVA model, $p=0.0066$) (Figure 4). Mean ($\pm$ SD) changes from baseline in salivary cortisol concentrations for male and female dogs were 0.117 ± 0.104 and 0.068 ± 0.099 μg/dL, respectively. Four-level day was not statistically significant ($p=0.7442$ when included as a factor in the ANCOVA model) so was excluded from analyses.

3.2 Behavioral data

3.2.1 Motor activity: There was a significant linear trend of decreased distance traveled over 5 days, based on an ANCOVA model including day as a covariate and period (pre-treatment, during treatment, post-treatment) as a factor (average decrease of 4.5 meters (m) per day, $p=0.0032$) suggesting that dogs habituated to the open field over time (Figure 5). The overall effect of period in the model was also significant ($p=0.0401$). Dogs tended to be more active when first entering the enclosure, with period A showing significantly greater distance traveled than C (mean change between A and C= 13.1 m, $p=0.0122$), and period B was not significantly greater than C (mean change between B and C= 4.9 m, $p=0.3480$). Periods A and B were not significantly different ($p=0.1133$).

Distance was further evaluated using an ANOVA model including day (as Day 1, Day 2, and Days 3-5) and period, to avoid the assumption of a linear relationship between days. Grouping was based on the apparent habituation to the open field. Significant differences in total distance traveled were present between the three groups of days (means: Day 1, 34.0 m; Day 2, 23.0 m; Days 3-5, 15.6 m; overall $p =0.0035$). The average distance traveled during Day 1 was significantly longer than the average of Days 3-5 ($p=0.0123$) but Days 1 and 2 and Days 2 and 3-5 were not significantly different from each other ($p=0.1027$ and 0.1731, respectively).

Treatment effects were also evaluated using ANOVA models. Treatment periods (Day 2B & Day 4B) were not significantly different than equivalent non-treatment periods (1B, 3B, 5B) in terms of average distance traveled (19.5 m vs. 19.7 m respectively, $p=0.9826$). No significant differences were found between thunderstorm treatment (Day 2B, 23.1 m) and gunfire treatment (Day 4B, 16.0 m), $p=0.5387$) or between thunderstorm vs. control ($p=0.7490$) and gunfire vs. control ($p=0.7152$).

When controlling for day and period, the following covariates were found to be significant across all days and periods in a multivariate backwards-stepping ANCOVA model: age (distance decreased 17.8 m per year of age [$p<0.0001$]), sex (males traveled 15.55m more than females [$p=0.0006$]).

3.2.2 Anxiety scores: Anxiety scores were assessed in three ways: positive, negative, and mean global scores (Table 2). Anxiety scores during the treatment periods (Days 2B & 4B) were not significantly different (positive $p=0.4297$, negative $p=0.3581$, mean global $p=0.3564$), so were grouped together for analysis purposes.

The effects of treatment on anxiety scores, after controlling for period and day, were evaluated using ANOVA models (Figures 6-8). Positive anxiety scores were significantly higher during treatment periods ($p<0.0001$), with scores also significantly higher during period A than C ($p=0.0469$). Negative anxiety scores were also significantly higher during treatment periods ($p=0.0099$), although there were no significant differences between periods A and C ($p=0.5998$). The same pattern held true for mean global anxiety (treatment effect, $p=0.0002$; difference between periods A and C, $p=0.1574$). An overall day effect was present in all models ($p<0.00001$, $p=0.0333$, and $p=0.0007$ respectively).

Dogs showed a range of behavioral responses in the open field reflected by their anxiety scores for each of the 3 domains. For individual dogs, these were presented as average change in anxiety during treatment periods (calculated as: [(score 2B - score 2A) + (score 4B – score 4A)]/2) for each dog.

4. Discussion

Habituation in an open field model is a well-documented phenomenon (Head 1997, Matsunaga 2010), and is an adaptive central nervous system phenomenon where a decreased response is seen to a continuous or repeated stimulus over time. This process may be seen within a session of exposure (intra-session habituation) or between sessions of exposure (inter-session habituation). In general, exploratory behavior in an open field is decreased both within a session and between sessions as the environment loses its novelty. This has been demonstrated in multiple species (Matsunaga 2010, Leussis 2006) including dogs (Head 1997). The current study looked at two physiologic measures: heart rate, and body temperature. The results of our study show that physiologic responses in the OFT are subject to a profound habituation response when measured at the start and end of the test session. The heart rate and body temperature changes seen in our study were reflective of a habituation response pattern. Post-session heart rates were higher on Day 1 as compared to other days, and there was a decrease in heart rate over the 9-minute course of the session on subsequent control days (Days 3 and 5). The lack of decrease in heart rate over the session on treatment days (Days 2 and 4) suggests that the sound stimuli did elicit a response, but that the test format was not sensitive enough to detect the response. The sound stimuli were presented during the middle three minutes of the session (period B), and it is possible that the dogs' heart rates were returning to baseline levels at the time of measurement (i.e., after Period C). Beerda (2008) found that recovery for heart rate increases following sound exposure in dogs was approximately 6 minutes from the onset of the noise stimulus. In future studies, a non-invasive measure for recording heart rate over the course of the session may better characterize heart rate variability during the test session (Bergamasco 2010).

Rectal temperature followed a similar pattern, with a slight increase in temperature over the course of the session (post-pre) on Day 1, but decreases or smaller increases occurring on the other days. As with heart rate, the use of a remote monitor that could evaluate temperature changes over the course of the session may have increased the sensitivity of this measure. However, rectal temperature may be more suitable as a measure of exercise stress and conditioning rather than psychological stress, and the changes seen in the present study may more closely mirror the changes in activity rather than emotional response (Piccione 2012). Sex differences in heart rate and body temperature were not observed in our study.

Salivary cortisol has been used as a non-invasive surrogate measure for plasma cortisol to detect the effects of stress in dogs (Beerda 2008, Haverbeke 2008). In general, salivary cortisol concentration increases as the HPA-axis is activated by a stressful event, such as a sound stimulus, and the resultant increase in plasma cortisol concentration may be reflected in the free fraction found in saliva (Kirschbaum 1989). In the present study, baseline saliva samples were taken at the same time of day as the OFT (Koleveska 2003) and were averaged to generate each individual's baseline result. Testing in the open field produced a significant increase in salivary cortisol concentration over baseline, although this value did not reveal an effect of session (Day). This may be due to insensitivity of the measure, or the use of the averaged baseline. In future work, saliva samples taken immediately pre- and post- session may reveal more contemporaneous changes. However, previous studies have supported the use of an averaged baseline measure (Haverbeke 2008, Kobelt 2003) and have not fully established circadian shifts in salivary cortisol levels in working dogs over a 24 hour period (Kolevska 2003). The lack of significant correlative finding is supported by Beerda (1998) in which there was a non-significant correlation between behavioral and physiological stress parameters in their tests of dogs exposed to different stimuli. Indeed, other efforts to establish the predictive validity of increased salivary cortisol levels in dogs have met with similar difficulty (Hekman 2012). Handling effects on salivary cortisol concentrations in this study are unlikely, since all samples were collected within 4 minutes of the end of a test session (Kobelt 2003) and dogs had been conditioned to the sample collection procedure. In the present study, there was an observed effect of sex for salivary cortisol concentration, with males having a higher salivary cortisol level than females.

Similar to the physiologic data, our OFT motor activity results suggest that dogs habituated to the open field over test days. We found that the dogs traveled significantly further on Day 1 than on subsequent days, and that they traveled further during the first three minutes (period A) than during the last three minutes. During the period of the sound stimulus presentation (period B – minutes 3-6), the mean distance traveled was not significantly different from the corresponding period during control days. This suggests that while motor activity may be a useful measure for evaluating habituation in the open field, it may not be a suitable model for anxiety, especially as individual dogs may show an increase or a decrease in activity as a result of anxiety (Haverbeke, 2008) and this may be masked by the profound effect of habituation on motor activity. Using a sound-stimulus open field model, Araujo (2013) found that dogs' overall activity was not affected by the sound stimuli, but their duration of inactivity was increased, suggesting that distance traveled alone is not sensitive enough to detect an anxiety response. This point is especially relevant to the current study, where blinded ratings of anxiety revealed increased anxiety during the stimulus periods, which was not revealed by the physiologic or motor activity results.

As predicted, we observed an increased expression of behaviors associated with fear and anxiety in dogs during the open field audio thunderstorm and simulated gun battle sessions, compared to control periods. Each dog was individually scored by a blinded observer on subjective but standardized measures of anxiety behaviors. There was no significant difference in dogs' behavioral responses to playback of the sound of either a thunderstorm or gun fire; however, both sound stimuli produced a measurable behavioral response. This finding highlights the need for careful observation of behavior to detect the effects of stress in MWDs, and is supported by findings in the literature (Beerda, 1998). Dependence on post-exposure physiologic or activity measures could result in an incorrect assumption that military working dogs do not incur psychological effects during stressful situations. In theater, IDDs may be exposed to loud, rapid, and extended fear-inducing sound stimuli, and the results of this study suggest that even less severe auditory stimuli may be sufficient to produce a measurable behavioral effect. In general, dogs recovered from the sound stimuli within a test session, with anxiety scores returning to pre-stimulus levels during Period C on both sound stimuli test days. Our study used only a 3 minute sound stimulus meant to generate a stress effect without producing distress (i.e., an aversive, negative state in which an animal's coping and adaptation responses fail to return the animal to a state of normal physiological and/or psychological well-being) (NAP, 2008). The effects of prolonged sound exposure were not investigated in this study; however our results suggest that even this relatively short exposure provides a meaningful increase in anxiety and a useful model for investigation of mitigation strategies. The return of anxiety scores to pre-sound stimuli levels also support the authors' assertion that real-time monitoring of physiologic data could prove meaningful.

Importantly, individual dogs were stratified relative to behavioral responses during sound stimuli (thunderstorm and gunfire). Using mean global anxiety as a composite representation of response, a range of behavioral responses, from minor to profound, was observed. This allows the model to be a useful adjunctive evaluation for anxiety in candidate IDDs. In a companion report (Sherman, 2013), results from the OFT anxiety scores were well correlated with a screening test for emotional resilience (discussed in Sherman, 2013).

Based on our results, we can conclude that the OFT is a useful model for evaluating anxiety reactions in MWDs. As expected, dogs in this study showed habituation in the open field, as measured by physiologic and activity data. However, the sound stimuli were sufficient to produce a measurable anxiety response in their observed behaviors. This allows the open field model, and the use of a standardized subjective scoring system to be applied to future studies using military working dogs, including the investigation of mitigation strategies for anxiety inducing events such as those that might be encountered in theater.

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Table 1. Open Field experimental protocol.

Period	Day 1 Session 1	Day 2 Session 2	Day 3 Session 3	Day 4 Session 4	Day 5 Session 5
Pre-Session	HR, RR, BT	HR, RR, BT	HR, RR, BT	HR, RR, BT	HR, RR, BT
A (3 min) in Open Field	No sound	No sound	No sound	No sound	No sound
B (3 min) in Open Field	No sound	Thunderstorm Sound	No sound	Gunfire Sound	No sound
C (3 min) in Open Field	No sound	No sound	No sound	No sound	No sound
Post-Session	HR, RR, BT *Salivary Cortisol	HR, RR, BT *Salivary Cortisol	HR, RR, BT *Salivary Cortisol	HR, RR, BT *Salivary Cortisol	HR, RR, BT *Salivary Cortisol

HR = Heart rate recorded
RR = Respiratory rate recorded
BT = Body temperature recorded
*Salivary Cortisol = Saliva sampled collected

Table 2. The scoring rubric for generation of anxiety scores.

Anxiety	**Expression of anxiety behaviors**
1	None; No anxiety behaviors
2	Occasional and mild
3	Some of the time and mild / Occasional and moderate
4	Most of the time and mild / Some of the time and moderate / Occasional and severe
5	Some of the time and severe / Most of the time and moderate
6	Most of the time and severe

Table 3. Definitions of anxiety score terminology

Anxiety Score Types	Definition
Negative (passive)	Passive behaviors, including decreased activity, such as freezing, hiding, position against wall, or at door; lowered body postures, such as crouching, tail tucking and ears back; and autonomic /conflict behaviors, such as panting, shaking, salivating, yawning, lip licking, or elimination.
Positive (active)	Positive anxiety behaviors included startling, bolting, vigilance, scanning, and active responses, such as pacing, aimless activity, stereotypic circling, retreat/escape attempts, digging, and climbing.
Global	Overall opinion of blinded rater, based on observation of dog and determination of negative and positive anxiety behaviors.
Mean Global	Numeric average of negative and positive scores

Figures

Figure 1. The open field test arena with "hide." The schematic representation, not to scale, shows the approximate location of the door, 2 elevated speakers. and 2 cameras. One camera was positioned overhead in the center of the arena, and one camera was laterally positioned, 0.6 m above the floor level, at a window just large enough to accommodate the camera lens.

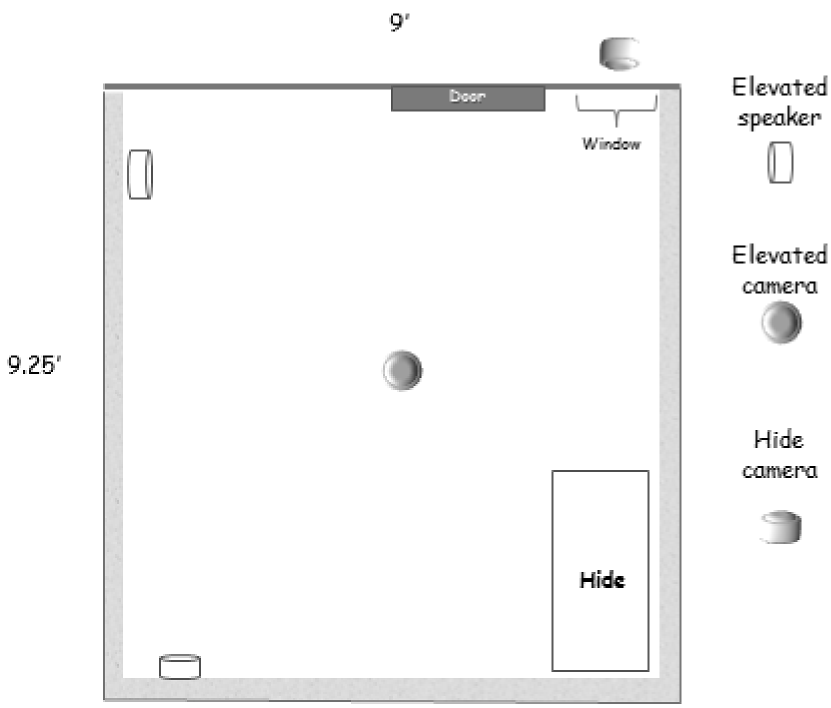
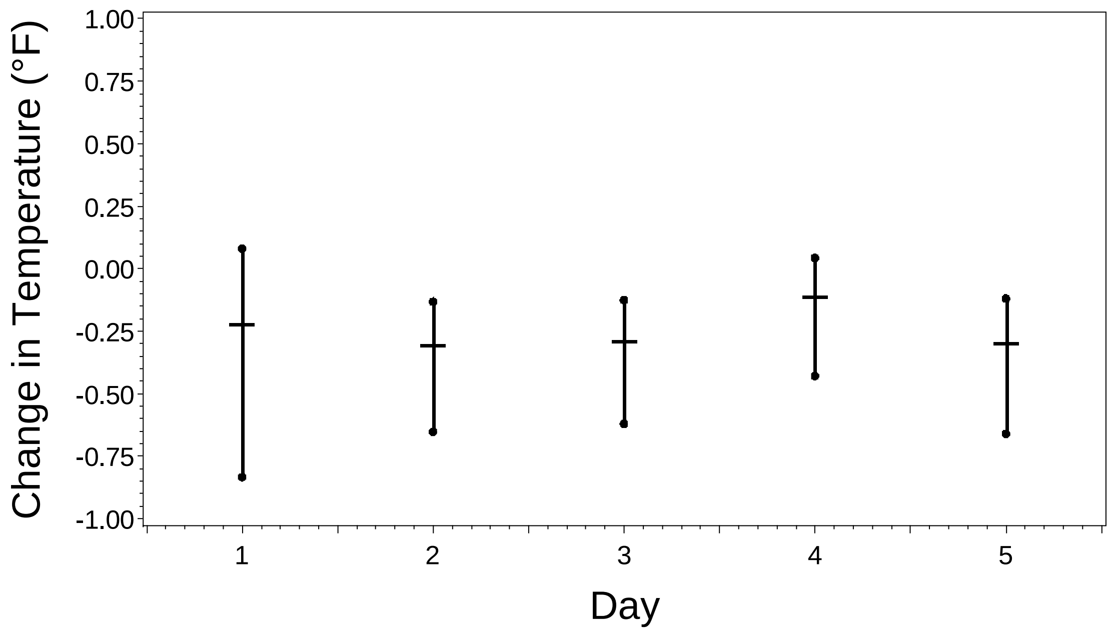


Figure 2. Mean (± SD) change in heart rate over days (post value – pre value) in the open field.



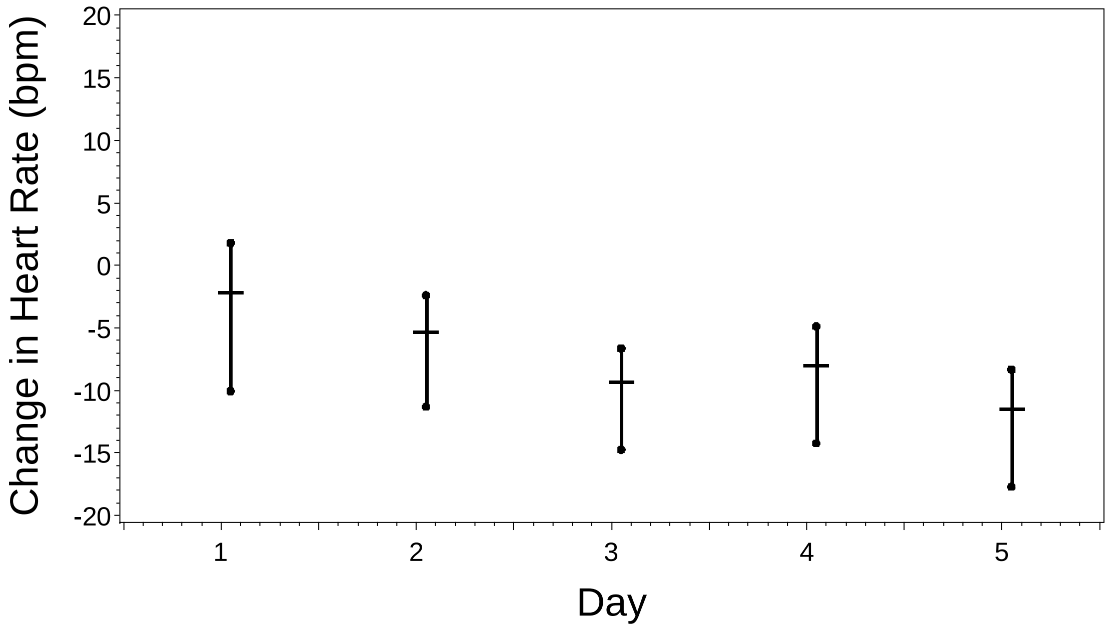


Figure 3. Mean (± SD) change in body temperature over days (post value – pre value) in the open field.

Figure 4. Mean (+/- SD) change in salivary cortisol (post value-baseline value) over days for the open field sessions for males (gray) and females (black).

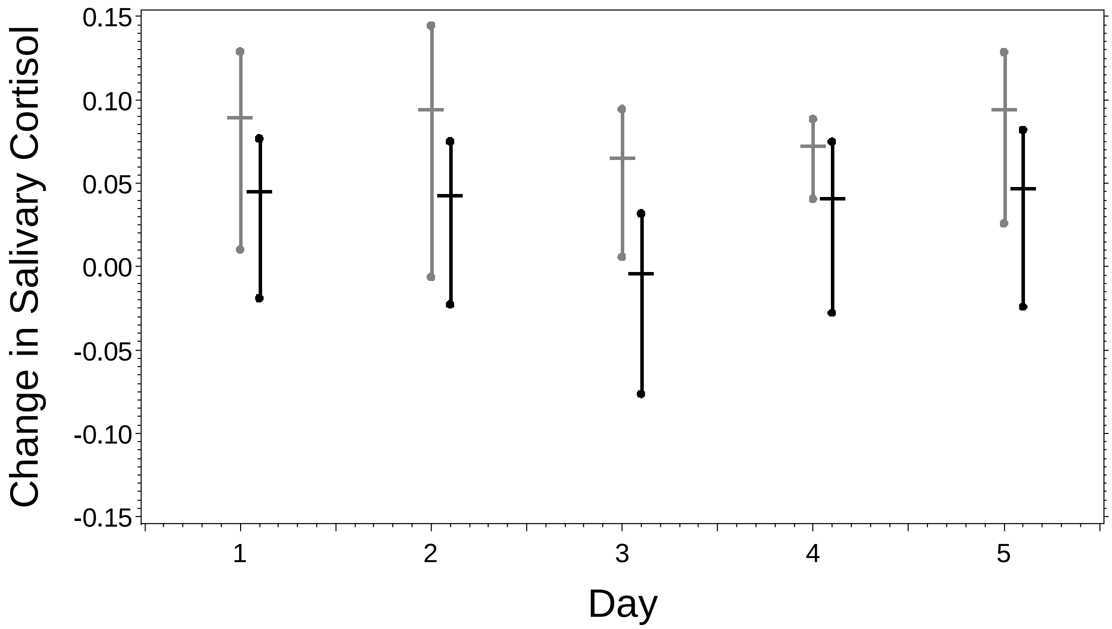


Figure 5. Mean (+/- SD) distance traveled (in meters) in the open field over days.

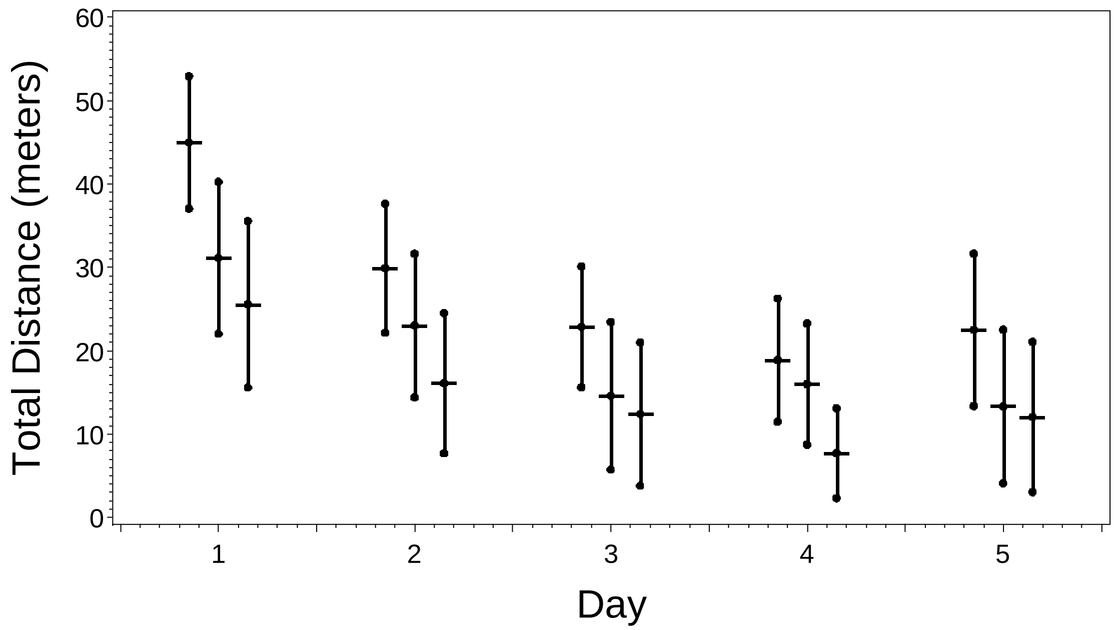


Figure 6. Mean (+/- SD) positive anxiety scores by Day and Period (A, B, C). On days 2 and 4, positive anxiety scores were higher in response to sound stimuli (Period B) compared to periods A and C (p<0.0001).

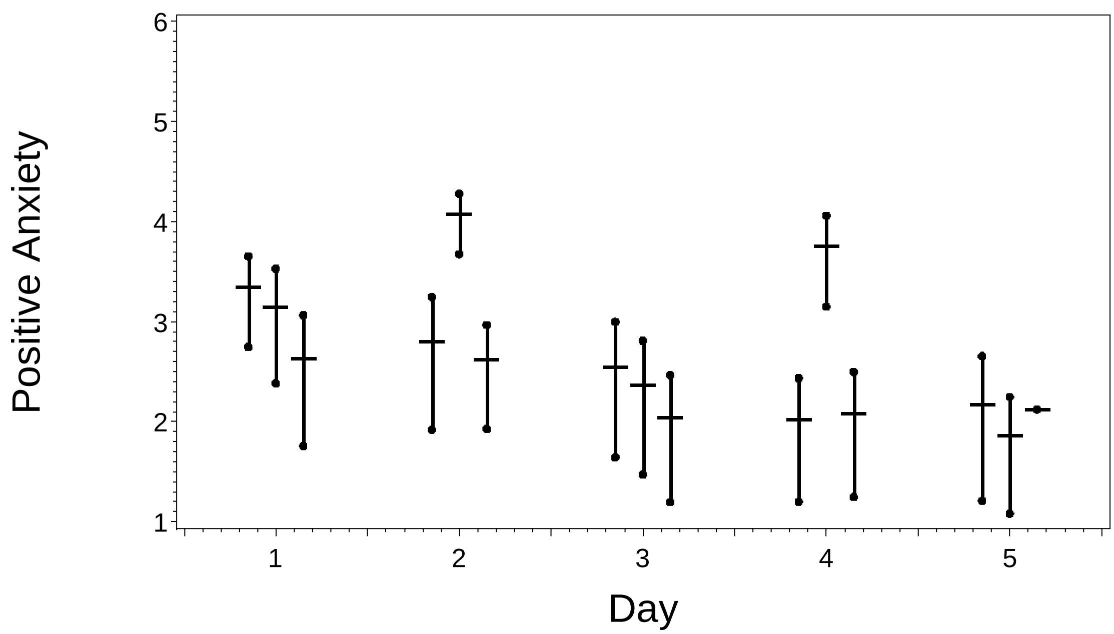


Figure 7. Mean (+/-SD) negative anxiety scores by Day and Period (A, B, C). On days 2 and 4, negative anxiety scores were higher in response to sound stimuli (Period B) compared to periods A and C (p<0.0099).

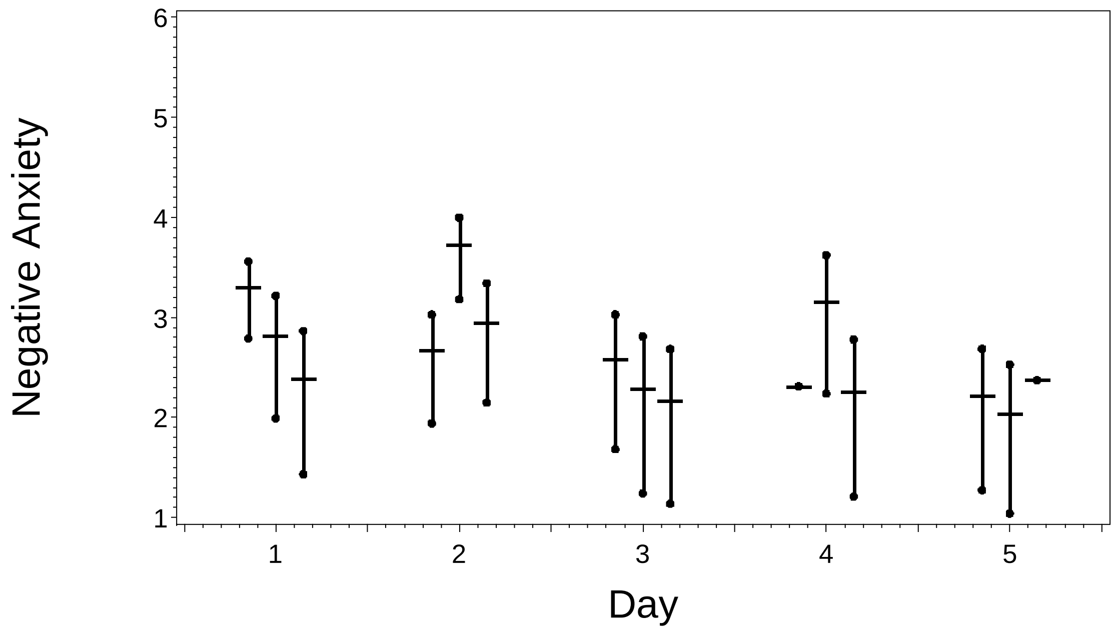


Figure 7. Mean (+/-SD) mean global anxiety scores by Day and Period (A, B, C). On days 2 and 4, mean global anxiety scores were higher in response to sound stimuli (Period B) compared to periods A and C ($p<0.0002$).